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Molecular Cloning, Expression and Characterization of A Novel Mouse SULT6 Cytosolic Sulfotransferase

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By searching the mouse EST database, we identified a novel mouse cytosolic sulfotransferase (SULT) cDNA (RIKEN cDNA 2410078J06). Sequence analysis revealed that this new SULT belongs to the cytosolic SULT6 gene family. The recombinant form of this newly identified SULT, designated SULT6B1, was expressed using the pGEX-4T-1 glutathione *S*-transferase fusion system and purified from transformed BL21 *Escherichia coli* cells. Purified mouse SULT6B1 exhibited sulfonating activity toward thyroxine and bithionol among a variety of endogenous and xenobiotic compounds tested as substrates. pH optimum of purified mouse SULT6B1 was determined to be 8.0. Tissue-specific expression of mouse and human SULT6B1 was examined by RT–PCR. While human SULT6B1 was specifically expressed in kidney and testis, mouse SULT6B1 was detected in brain, heart, kidney, thymus, lung, liver and testis. Further studies are needed in order to clarify the role of SULT6B1 in the metabolism of thyroxine and possibly some xenobiotics in mouse.

Key words: detoxification, metabolism, sulfonation, sulfotransferase, thyroxine.

Abbreviations: 5′-PSB motif, 5′-phosphosulfate binding motif; 3′-PB motif, 3′-phosphate binding motif; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; RT–PCR, reverse transcription–polymerase chain reaction; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SULT, sulfotransferase.

In mammals, the cytosolic sulfotransferases (SULTs) are a group of enzyme that catalyse the transfer of a sulfonate group from the active sulfate, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), to a variety of endogenous and xenobiotic compounds containing hydroxyl and/or amino group (*1*). These enzymes are generally thought to be involved in the biotransformation/excretion of drugs and xenobiotics, as well as in the regulation/homeostasis of endogenous compounds such as catecholamines, cholesterol, steroids, thyroid hormones and bile acids in mammals (*2*–*4*).

All cytosolic SULTs constitute a gene superfamily (*5*). Based on the amino acid sequences of known cytosolic SULTs, six distinct gene families have been classified. Subfamilies within some of the SULT gene families have been further categorized. It is generally accepted that members of the same SULT gene family share at least 45% amino acid sequence identity, while members of a subfamilies further divided within each SULT gene family show >60% identity in amino acid sequence (*6*, *7*). The SULT1 family presently consists of five subfamilies: phenol SULT (SULT1A) (*8*), Dopa/tyrosine (or thyroid hormone) SULT (SULT1B) (*9*), hydroxylamine (or acetylaminofluorene) SULT (SULT1C) (*10*), tyrosine ester SULT (SULT1D) (*11*) and oestrogen SULT (SULT1E) (*12*). The SULT2 family comprises two subfamilies, namely SULT2A and SULT2B (*11*, *13*, *14*). Isoforms within each of the two SULT2 subfamilies catalyse the sulfonation of the 3β-hydroxyl groups of steroids with unsaturated 'A' rings, e.g. dehydroepiandrosterone (DHEA). Members of the SULT3 family catalyses the sulfonation of heterocyclic amines such as desipramine (*15*, *16*). Members of the SULT4 family have been identified in mouse, rat and human, and little is known about these highly conserved SULTs that are expressed specifically in brain and other neuronal tissues (*17*). Another SULT that belongs to the SULT5 family has been cloned from mouse, but no information concerning its function is currently available (*5*). Lastly, a novel chicken cytosolic SULT that catalyses the sulfonation of 17β-estradiol and corticosterone, has been identified (*18*) and proposed to belong to the SULT6 family.

In this communication, we report the molecular cloning, expression and characterization of a novel mouse cytosolic SULT6 enzyme. The enzymatic activity toward endogenous compounds and a variety of xenobiotic compounds was examined. Moreover, its tissue-specific expression in different mouse organs was investigated by RT–PCR analysis.

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MATERIALS AND METHODS

Materials—Bithionol and corticosterone were purchased from Wako Pure Chemical Industries, Ltd. Thyroxine, 3,3′,5′-triiodo-L-thyronine, 3,3′-diiodo-L-thyronine, 3-iodo-L-tyrosine, bisphenol A, 17β-estradiol and 1,3-bis[tris(hydroxymethyl)methylamino]propane (bis-tris propane) were products of Sigma Chemical Company. 3,3′,5-triiodo-L-thyronine sodium salt was obtained from MP biomedicals, Inc. ReverTra Ace, KOD-plus- and all restriction endonucleases were from TOYOBO. Mouse total RNAs obtained from OriGene Technologies were used. This RNAs included mixture of three male and female mice per organs/tissues, except for testis. Human total RNAs purchased from Clontech included one or more than three human male, female or male/female per organs/tissues per lot: brain (male), heart (10 male/female), kidney (female), lung (3 male/female), thymus (3 male), liver (male) and testis (39 male). Isopropyl β-D-thiogalactopyranoside (IPTG) and *TaKaRa Ex Taq* Hot Start Version were purchased from TAKARA. T4 DNA ligase was from New England Biolabs. Oligonucleotide primers were synthesized by NIPPON EGT and Hokkaido System Science. pBluescript II SK(+) and XL1-Blue MRF' and BL21 *Escherichia coli* host strain were from Stratagene. pGEX-4T-1 prokaryotic GST fusion vector and glutathione Sepharose 4B were products of GE Healthcare Biosciences. Cellulose and Silica gel thin-layer chromatography (TLC) plates were from Merck. All other chemicals were of the highest grade commercially available.

Molecular Cloning of Novel Cytosolic SULT—RT–PCR technique was employed to amplify the new mouse SULT nucleotide sequence. Mouse heart total RNA was purchased from OriGene Technologies. First-strand cDNA was synthesized using First-Strand cDNA Synthesis Kit (TOYOBO) with oligo(dT) as primer. PCR in a 20 μl reaction mixture was carried out under the action of KOD-plus-; with mouse heart first-strand cDNA as the template in conjunction with gene-specific sense and antisense oligonucleotide primers (Table 1). Amplification conditions were 10 cycles of 30 s at 95°C, 30 s at 60 to 50°C (a decrease of 1°C per cycle), 1.5 min at 68°C, followed by 25 cycles of 30 s at 95°C, 30 s at 50°C, 1.5 min at 68°C. The final reaction mixture was applied onto a 1.2% agarose gel, separated by electrophoresis and visualized by ethidium bromide staining. Bands corresponding to discrete PCR products (~950 bp in size) from the PCR reaction was excised from the gel, and the DNA fragments therein was isolated by spin filtration. Purified PCR products was individually subcloned into the *Bam*HI/*Xho*I site of pBluescript II SK(+) and transformed into *E. coli* XL1-Blue MRF'. To verify its authenticity, the cDNA inserts was subjected to nucleotide sequencing (*19*). Upon verification, the insert was released by *Bam*HI/*XhoI* digestion, isolated by spin filtration and subcloned into the *Bam*HI/*Xho*I site of pGEX-4T-1 prokaryotic expression vector.

Bacterial Expression and Purification of the Recombinant Mouse Cytosolic SULT—pGEX-4T-1 harboring the newly cloned mouse *SULT* cDNA was transformed into competent *E. coli* BL21 cells. Transformed BL21 cells were grown to $OD_{600\ nm}$ = ~0.2 in 100 ml LB medium supplemented with 100 μg/ml ampicillin and induced with 0.1 mM IPTG. After a 4 h induction at 22.5°C, the cells were collected by centrifugation and homogenized in 25 ml ice-cold lysis buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl and 1 mM EDTA) using an Ohtake French Press. The crude homogenate was subjected to centrifugation at 20,400*g* for 25 min at 4°C. The supernatant collected was fractionated using 0.5 ml of glutathione Sepharose 4B, and the bound GST fusion protein was treated with 1 ml of a thrombin digestion buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl and 2.5 mM $CaCl_2$) containing 5 unit/ml bovine thrombin. Following an overnight digestion on ice, the preparation was subjected to centrifugation.

Enzymatic Assay—The sulfotransferase activity was assayed using PAP[^{35}S] as the sulfate donor. The standard assay mixture, with a final volume of 25 μl, contained 50 mM bis-tris propane buffer (pH 8.0), 0.5 μM PAP[^{35}S] (45 Ci/mmol), 100 μM substrate and 2.5 μg purified recombinant enzyme. For the kinetic studies on the sulfonation of thyroxine, 10, 20, 40, 50, 80 μM of thyroxine, 0.5 μM PAP[^{35}S] and 50 mM bis-tris propane buffer at pH8.0 were used. The reaction was started by the addition of the enzyme, allowed to proceed for 30 min at 37°C, and terminated by heating at 100°C for 3 min. The precipitates formed were cleared by centrifugation, and the supernatant was subjected to the analysis of [^{35}S]sulfated product using the previously developed thin-layer chromatography separation

Table 1. **Oligonucleotide primers used for the cDNA cloning of mouse SULT6B1 and for RT–PCR.** Analysis of the tissue-specific expression of mouse SULT6B1 and human SULT6B1.

For cDNA cloning of mouse SULT6B1 cDNAs		
Mouse SULT6B1	Sense	5′-CGCGGATCCATGGCTGATAAGTCCAAGTTTATCG-3′
	Antisense	5′-GGTGCTCGAGAGCCAGGCAATATGCTTCAT-3′
For RT–PCR analysis		
Mouse SULT6B1	Sense	5′-GGAAACTTTCCGAGCTTTGGA-3′
	Antisense	5′-GTGCTCTGAGTGGAGATGGT-3′
Mouse 18S rRNA	Sense	5′-CTTAGAGGGACAAGTGGCG-3′
	Antisense	5′-TACACTGACTGGCTCAGCGT-3′
Human SULT6B1	Sense	5′-GCCATGCGTGCGAAGTCT-3′
	Antisense	5′-CTTCACCTTTGCGGAAAAGG-3′
Human β-actin	Sense	5′-CTGGAACGGTGAAGGTGACA-3′
	Antisense	5′-AAGGGACTTCCTGTAACAATGCA-3′

procedure (*20*), with *n*-butanol/isopropanol/formic acid/water (3:1:1:1; by volume) as the solvent system. Afterwards, the plates were air-dried and analysed using a Fluoro Image Analyzer (FLA-3000G). It should be pointed out that this method has a detection limit of 0.5 pmol/min/mg. Therefore, any activity below this level was marked as ND (activity not detected).

Analysis of the Tissue-specific Distributions of Novel SULTs—For use as templates in RT–PCR, first-strand cDNAs were reverse-transcribed from total RNAs isolated from different mouse and human tissues/organs using random hexamers according to the manufacturer's instruction (TOYOBO). Oligonucleotide primers for RT–PCR-amplification of mouse SULT6B1 and human SULT6B1 cDNAs were designed using the Primer Express software (Applied Biosystems). Using sense (5′-GGAAACTTTCCGAGCTTTGGA-3′) and antisense (5′-GTGCTCTGAGTGGAGATGGT-3′) oligonucleotide primers for mouse SULT6B1, and sense (5′-GCCAT GCGTGCGAAGTCT-3′) and antisense (5′-CTTCACCTTT GCGGAAAAGG-3′) oligonucleotide primers for human SULT6B1, PCR reactions in 20 μl reaction mixtures were carried out under the action of *TaKaRa Ex Taq* Hot Start Version, with each of the seven different (brain, heart, kidney, thymus, lung, liver and testis) first-strand cDNAs prepared as template. Reaction conditions were 3 min at 95°C for initial denaturation, followed by 30 cycles of 30 s at 95°C, 30 s at 55°C and 30 s at 72°C. The final reaction mixtures were applied onto a 2.0% agarose gel, separated by electrophoresis, and visualized by ethidium bromide staining.

Miscellaneous Methods—PAP[^{35}S] (45 Ci/mmol) was synthesized from ATP and [^{35}S]sulfate using recombinant human bifunctional ATP sulfurylase/adenosine 5′-phosphosulfate kinase as described previously (*21*). SDS–PAGE was performed on 12% polyacrylamide gels using the method of Laemmli (*22*). Protein determination was based on the method of Lowry with bovine serum albumin as the standard (*23*).

RESULTS AND DISCUSSION

With the completion of mouse genome sequencing (*24*), the mouse is becoming an ever useful animal model for various studies. As part of our long standing interest in using the mouse to study the functional relevance of the cytosolic SULTs, we recently identified a novel cytosolic SULT cDNA (RIKEN cDNA 2410078J06; designated as mouse SULT6B1) from the mouse EST database. Further analysis revealed that the gene encoding this novel cytosolic SULT, designated mouse SULT6B1, is localized on chromosome 17 and mapped to the E3 region. Mouse SULT6B1 has a genome structure very similar to human SULT6B1 (*25*). Here, we report the cloning, expression, and characterization of this novel mouse cytosolic SULT.

Molecular Cloning of Novel Mouse Cytosolic SULT—The mouse SULT6B1 cDNA cloned by RT-PCR was subjected to nucleotide sequencing. Fig. 1 shows the deduced amino acid sequences of SULT6B1. The open reading frame encompasses 909 nucleotides and encodes a 303 amino acid polypeptide. Similar to other cytosolic SULTs, the new mouse SULT6B1 contains sequences resembling the so-called '5′-phosphosulfate binding (5′-PSB) motif' (YPKxGxxW in the N-terminal region) and '3′-phosphate binding (3′-PB) motif' (VxxRNPKDxxVSxFH in the central region) (*26*) which have been proposed to be involved in the binding of PAPS, a co-substrate for the SULT-catalysed sulfonation reactions (*1*). Sequence analysis revealed that the

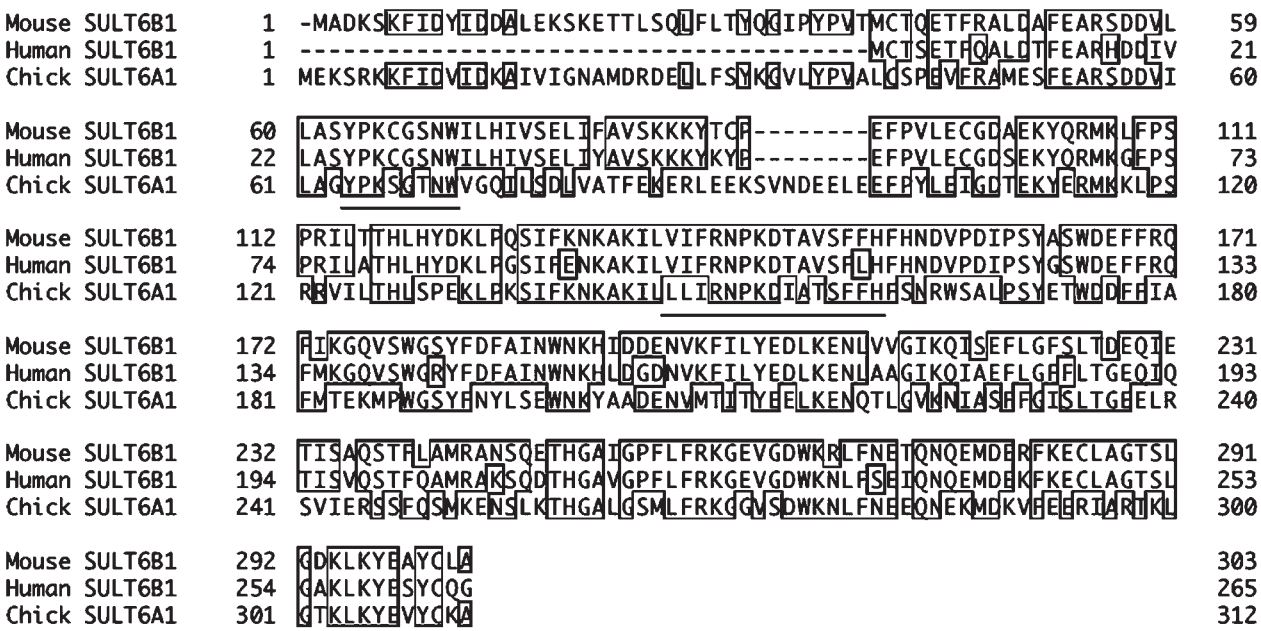

Fig. 1. **Alignment of the deduced amino acid sequences of mouse SULT6B1, human SULT6B1 and chicken SULT6A1.** Residues conserved among at least two of the three enzymes are boxed. 5′-PSB motif and 3′-PB motif located, respectively, in the N-terminal and middle region are underlined.

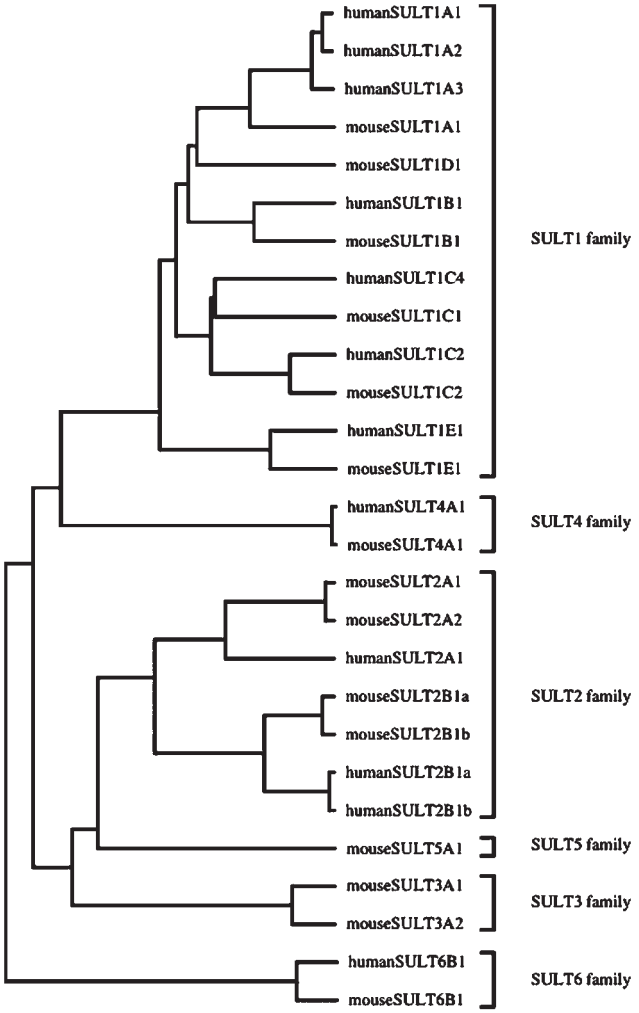

Fig. 2. **Classification of the mouse and human SULT6B1 on the basis of their amino acid sequences.** The dendrogram shows the degree of amino acid sequence homology among cytosolic SULTs. See review by Blanchard *et al*. (*5*) for individual SULTs references.

deduced amino acid sequence of the mouse SULT6B1 displays 32.6, 31.8, 30.1, 29.8, 26.7, 28.3, 85.6 and 52.0% amino acid sequence identity to mouse SULT1A1, SULT1B1, SULT2A1, SULT3A1, SULT4A1, SULT5A1, human SULT6B1 and chicken SULT6A1, respectively. It is generally accepted that members of the same SULT gene family share at least 45% amino acid sequence identity, whereas members of subfamilies further divided within each SULT gene family are greater than 60% identical in amino acid sequence (*6*,*7*). Based on these criteria, the newly cloned mouse SULT clearly belongs to the SULT6 gene family that currently includes only the human SULT6B1 (Fig. 2) (*5*).

Expression, Purification and Characterization of Recombinant Mouse SULT—The coding sequence of the mouse SULT6B1 was subcloned into pGEX-4T-1, a prokaryotic expression vector, for the expression of the recombinant enzyme in *E. coli*. Purified recombinant mouse SULT6B1 was subjected to functional

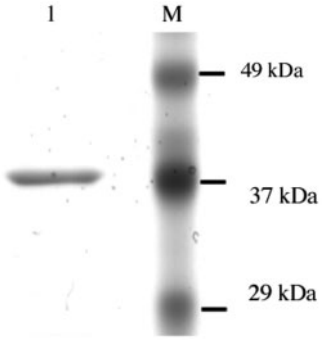

Fig. 3. **SDS gel electrophoretic patterns of recombinant mouse SULT6B1.** Samples were subjected to SDS–PAGE on a 12% gel, followed by Coomassie blue staining. Lane 1 corresponds to the purified enzyme. Protein molecular weight markers co-electrophoresed were myoglobin (M_r = 29,000), carbonic anhydrase (M_r = 37,000) and ovalbumin (M_r = 49,000).

Table 2. **Specific activities of the mouse SULT6B1 with endogenous and xenobiotic compounds as substrates.**[a]

	10 μM	100 μM
Thyroxine	4.2 ± 0.3	9.6 ± 0.5
3,3′,5-Triiodothyronine	ND[b]	ND
3,3′,5′-Triiodothyronine	ND	ND
3,3′-Diiodothyronie	ND	ND
3-Iodotyrosine	ND	ND
Bithionol	7.5 ± 0.5	7.9 ± 0.4
Bisphenol A	ND	ND
p-Nitrophenol	ND	ND
1-Naphthylamine	ND	ND
17β-Estradiol	ND	ND
Corticosterone	ND	ND

[a]Specific activity refers to picomole of sulfonated product formed per minute per milligram. Data shown represent the mean ± SD from three determinations. [b]ND, not detected (specific activity determined was lower than the detection limit estimated to be ~0.5 pmol/min/mg protein).

characterization with respect to its sulfonating activity. As shown in Fig. 3, the purified recombinant mouse SULT6B1 migrated as a single 37 kDa protein band upon SDS–PAGE under reducing condition. This latter result is in line with a molecular mass of 36.46 kDa determined based on the MALDI-TOF MS analysis. A number of endogenous and xenobiotic compounds were first tested as substrates for the purified mouse SULT6B1. The activity data obtained are compiled in Table 2. Among the substrates we tested, mouse SULT6B1 displayed sulfonating activities toward thyroxine and bithionol. Purified mouse SULT6B1 showed no sulfonating activity toward 3,3′,5-triiodothyronine, 3,3′,5′-triiodothyronine, 3,3′-diiodothyronine, 3-iodotyrosine, *p*-nitrophenol and 1-naphthylamine used. However, mouse SULT6B1 has not concentration-dependency for bithionol, raising the possibility that sulfonating activity for bithionol at those concentration reached saturation level (Table 2). It was also suggested that, mouse SULT6B1 exhibited substrate inhibition with higher concentration of bithionol. It is known that the enzymatic reactions of SULTs require PAPS as a co-factor. The intracellular PAPS concentration *in vivo* had been

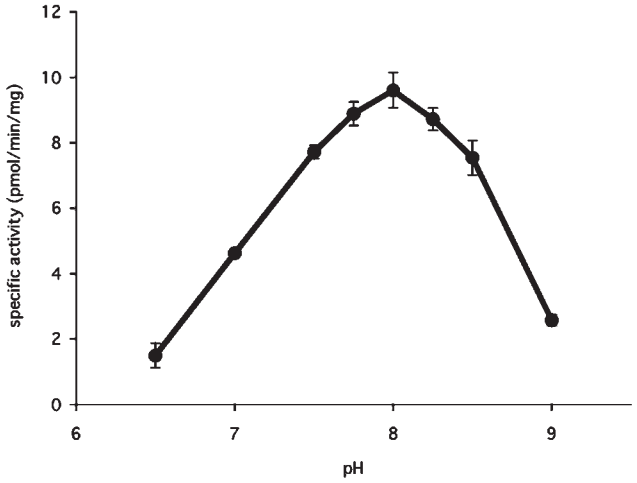

Fig. 4. **pH-dependence of the recombinant mouse SULT6B1 activity with 100 μM thyroxine as the substrate.** The enzymatic assay was carried out under standard assay conditions as described in MATERIALS AND METHODS section, except that buffers with different pH values were used was 50 mM bis-tris propane. The data represents the mean ± SD derived from three independent experiments.

Table 3. **Kinetic constant of the mouse SULT6B1 with thyroxine as substrate.**

	K_m (μM)	V_{max} (pmol/min/mg)	V_{max}/K_m
Thyroxine	143.7	40.4	0.3

demonstrated to be critical to the extent of sulfonation of different substrates (*27*). How the PAPS concentration may affect the sulfonation of thyroxine, bithionol and perhaps other substrates, by mouse SULT6B1, however, remains an open question. A pH-dependence experiment was subsequently carried out using 100 μM thyroxine as the substrate. Mouse SULT6B1 displayed a pH optimum at 8.0 with thyroxine as the substrate (Fig. 4). Activity data showed that mouse SULT6B1 was active toward thyroxine and bithionol only at pH 7.5 and 8.0, with higher activity detected at pH 8.0 than at pH 7.5. It is noted that, thyroxine was the only endogenous substrate used by the mouse SULT6B1 (Table 2). Interestingly, although chick SULT6A1 displayed sulfonation activity toward 17β-estradiol and corticosterone (*18*), mouse SULT6B1 was inactive toward these substrates. Table 3 shows the kinetic constant determined for the sulfonation of thyroxine by mouse SULT6B1. Data obtained were processed using the Excel program to generate the best fitting trendlines for the Lineweaver–Burk plots. In comparison with other SULTs, mouse SULT6B1 displayed higher K_m with thyroxine as substrate (*28*).

Thyroxine is a prohormone secreted by the thyroid gland which has little or no biological activity. Thyroxine is converted to 3,3′,5-triiodothyronine in peripheral tissues by 5′-deiodination. Both thyroxine and 3,3′,5-triiodothyronine are inactivated by 5-deiodination to 3,3′,5′-triiodothyronine and 3,3′-diiodothyronine, respectively (*29*). Under normal conditions, 5′-deiodination and 5-deiodination were predominant pathways of

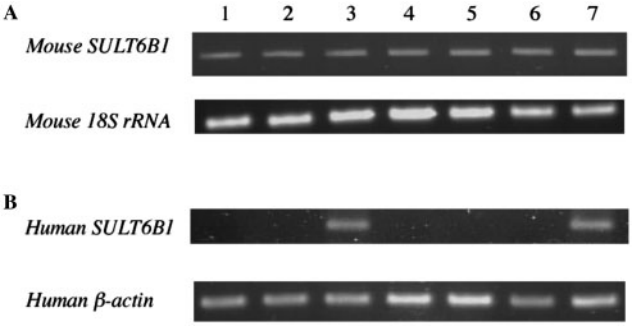

Fig. 5. **RT–PCR analysis of the expression of mouse and human SULT6B1 in different tissues/organs.** (A) Expression level of mouse SULT6B1 mRNA and 18S rRNA were analysed by RT–PCR as described in MATERIALS AND METHODS section. Lane 1, brain; lane 2, heart; lane 3, kidney; lane 4, thymus; lane 5, lung; lane 6, liver; lane 7, testis. (B) RT–PCR analysis of the expression of the human SULT6B1 and β-actin mRNA in the same tissues as described in (A).

thyroxine metabolism. However, if deiodination was saturated at high substrate concentrations, conjugates including thyroxine-sulfate (*30*) were found to accumulate. Thyroxine-sulfate is not converted to 3,3′,5-triiodothyronine-sulfate and is inactivated by sulfonation (*29*). Previous studies have demonstrated mammalian cytosolic SULTs exhibited sulfonating activity toward thyroid hormones (*9*,*17*), but none of them is specific for thyroxine. Our current data indicated that mouse SULT6B1 is uniquely specific for thyroxine, implying a role of this novel SULT in regulation of thyroxine.

Tissue-specific Expression of Mouse and Human SULT6B1—We were interested in examining the expression patterns of mouse and human SULT6B1 in different tissue/organs. RT–PCR was employed to examine the expression of mRNAs encoding mouse and human SULT6B1 in seven organs/tissues (see the MATERIALS AND METHODS section). Results showed that, although human SULT6B1 was specifically expressed in kidney and testis, mouse SULT6B1 was detected in brain, heart, kidney, thymus, lung, liver and testis (Fig. 5). Preliminary examination using total RNAs prepared in our laboratory showed that the expression pattern of mouse SULT6B1 did not differ between male and female tissue/organs (brain, heart, kidney, lung, liver, uterus and testis). Interestingly, mouse uterus and testis were found to express similar levels of mouse SULT6B1 (data not shown). In view of that the thyroid hormone transporter is expressed in the many tissues, it is possible that SULT6B1 expression in the seven mouse tissues may have relevance to the regulation of thyroxine uptake and metabolism. A recent study demonstrated that SULT mRNA expression level may be affected differences in age and sexual dimorphism (*31*). It is possible that differences between mouse and human SULT6B1 mRNA expression may be due to age and sexual dimorphism.

In conclusion, we have cloned, expressed and characterized a novel mouse cytosolic SULT6B1. Recombinant mouse SULT6B1, expressed in and purified from *E. coli*

cells, exhibited some distinct enzymatic properties including pH optimum and substrate specificity. RT–PCR analysis showed that mouse and human SULT6B1 displayed distinct patterns of expression in different tissues/organs. Further studies are warranted in order to unequivocally clarify the role of SULT6B1 in the metabolism of thyroxine and possibly some xenobiotics in mouse.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Lipmann, F. (1958) Biological sulfate activation and transfer. *Science* **128**, 575–580
2. Mulder, G.J. and Jakoby, W.B. (1990) *Conjugation Reactions in Drug Metabolism* (Mulder, G.J., ed.), pp. 107–161, Taylor and Francis Ltd, London
3. Falany, C. and Roth, J.A. (1993) *Human Drug Metabolism: From Molecular Biology to Man* (Jeffery, E.H., ed.), pp. 101–115, CRC Press, Inc., Boca Raton
4. Weinshilboum, R. and Otterness, D.M. (1994) *Conjugation-Deconjugation Reactions in Drug Metabolism and Toxicity* (Kaufmann, F.C., ed.), pp. 45–78, Springer, Inc., Berlin
5. Blanchard, R.L., Freimuth, R.R., Buck, J., Weinshilboum, R.M., and Coughtrie, M.W.H. (2004) A proposed nomenclature system for the cytosolic sulfotransferase (SULT) superfamily. *Pharmacogenetics* **14**, 199–211
6. Yamazoe, Y., Nagata, K., Ozawa, S., and Kato, R. (1994) Structural similarity and diversity of sulfotransferases. *Chem. Biol. Interact.* **92**, 107–117
7. Weinshilboum, R.M., Otterness, D.M., Aksoy, I.A., Wood, T.C., Her, C.T., and Raftogianis, R.B. (1997) Sulfation and sulfotransferases 1: sulfotransferase molecular biology: cDNAs and genes. *FASEB J.* **11**, 3–14
8. Ozawa, S., Nagata, K., Gong, D.W., Yamazoe, Y., and Kato, R. (1990) Nucleotide sequence of a full-length cDNA (PST-1) for aryl sulfotransferase from rat liver. *Nucleic Acids Res.* **18**, 4001
9. Sakakibara, Y., Takami, Y., Zwieb, C., Nakayama, T., Suiko, M., Nakajima, H., and Liu, M.-C. (1995) Purification, characterization, and molecular cloning of a novel rat liver Dopa/tyrosine sulfotransferase. *J. Biol. Chem.* **270**, 30470–30478
10. Nagata, K., Ozawa, S., Miyata, M., Shimada, M., Gong, D.W., Yamazoe, Y., and Kato, R. (1993) Isolation and expression of a cDNA encoding a male-specific rat sulfotransferase that catalyses activation of *N*-hydroxy-2-acetylaminofluorene. *J. Biol. Chem.* **268**, 24720–24725
11. Sakakibara, Y., Yanagisawa, K., Takami, Y., Nakayama, T., Suiko, M., and Liu, M.-C. (1998) Molecular cloning, expression, and functional characterization of novel mouse sulfotransferases. *Biochem. Biophys. Res. Commun.* **247**, 681–686
12. Nash, A.R., Glenn, W.K., Moore, S.S., Kerr, J., Thompson, A.R., and Thompson, E.O. (1988) Oestrogen sulfotransferase: molecular cloning and sequencing of cDNA for the bovine placental enzyme. *Aust. J. Biol. Sci.* **41**, 507–516
13. Otterness, D.M., Wieben, E.D., Wood, T.C., Watson, W.G., Madden, B.J., McCormick, D.J., and Weinshilboum, R.M. (1992) Human liver dehydroepiandrosterone sulfotransferase: molecular cloning and expression of cDNA. *Mol. Pharmacol.* **41**, 865–872
14. Her, C., Wood, T.C., Eichler, E.E., Mohrenweiser, H.W., Ramagli, L.S., Siciliano, M.J., and Weinshilboum, R.M. (1998) Human hydroxysteroid sulfotransferase SULT2B1: two enzymes encoded by a single chromosome 19 gene. *Genomics* **53**, 284–295
15. Yoshinari, K., Nagata, K., Ogino, M., Fujita, K., Shiraga, T., Iwasaki, K., Hata, T., and Yamazoe, Y. (1998) Molecular cloning and expression of an amine sulfotransferase cDNA: a new gene family of cytosolic sulfotransferase in mammals. *J. Biochem.* **123**, 479–486
16. Takahashi, S., Sakakibara, Y., Mishiro, E., Kouriki, H., Nobe, R., Kurogi, K., Yasuda, S., Liu, M.-C., and Suiko, M. (2008) Molecular cloning, expression, and characterization of mouse amine *N*-sulfotransferases. *Biochem. Biophys. Res. Commun.* **375**, 431–435
17. Sakakibara, Y., Suiko, M., Pai, T.G., Nakayama, T., Takami, Y., Katafuchi, J., and Liu, M.-C. (2002) Highly conserved mouse and human brain sulfotransferases: molecular cloning, expression, and functional characterization. *Gene* **285**, 39–47
18. Cao, H., Agarwal, S.K., and Burnside, J. (1999) Cloning and expression of a novel chicken sulfotransferase cDNA regulated by GH. *J. Endocrinol.* **160**, 419–500
19. Sanger, F., Nicklen, S., and Coulson, A.R. (1977) DNA sequencing with chain-terminating inhibitors. *Proc. Natl Acad. Sci. USA* **74**, 5463–5467
20. Liu, M.-C. and Lipmann, F. (1984) Decrease of tyrosine-*O*-sulfate-containing proteins found in rat fibroblasts infected with Rouse sarcoma virus or Fujinami sarcoma virus. *Proc. Natl Acad. Sci. USA* **81**, 3695–3698
21. Yanagisawa, K., Sakakibara, Y., Suiko, M., Takami, Y., Nakayama, T., Nakajima, H., Takayanagi, K., Natori, Y., and Liu, M.-C. (1998) cDNA cloning, expression, and characterization of the human bifunctional ATP sulfurylase/adenosine 5′-phosphosulfate kinase enzyme. *Biosci. Biotechnol. Biochem.* **62**, 1037–1040
22. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685
23. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**, 265–275
24. Mouse Genome Sequencing Consortium. (2002) Initial sequencing and comparative analysis of the mouse genome. *Nature* **420**, 520–562
25. Freimuth, R.R., Wiepert, M., Chute, C.G., Wieben, E.D., and Weinshilboum, R.M. (2004) Human cytosolic sulfotransferases database mining: identification of seven novel genes and pseudogenes. *Pharmacogenomics J.* **4**, 54–65
26. Kakuta, Y., Pedersen, L.G., Pedersen, L.C., and Negishi, M. (1998) Conserved structural motifs in the sulfotransferase family. *Trends Biochem. Sci.* **23**, 129–130
27. Klaassen, C.D. and Boles, J.W. (1997) The importance of 3′-phosphoadenosine 5′-phosphosulfate (PAPS) in the regulation of sulfation. *FASEB J.* **11**, 404–418

28. Li, X., Clemens, D.L., and Anderson, R. J. (2000) Sulfation of iodothyronines by human sulfotransferase 1C1 (SULT1C1)*. *Biochem. Pharmacol.* **60**, 1713–1716
29. Visser, T.J. (1988) Metabolism of thyroid hormone in *Hormones and their Actions Part I in New Comprehensive Biochemistry* (Cooke, B.A., King, R.J.B., and van der Molen, H.J., eds) Vol. 18A, pp. 81–103, Elsevier science, Amsterdam, The Netherlands
30. Sato, K. and Robbins, J. (1981) Thyroid hormone metabolism in primary cultured rat hepatocytes; effects of glucose, glucagons, and insulin. *J. Clin. Invest.* **68**, 475–483
31. Alnouti, Y. and Klaassen, C. D. (2006) Tissue distribution and ontogeny of sulfotransferase enzymes in mice. *Toxicol. Sci.* **93**, 242–255